

e.g., diluent, binding agent, disintegrant, etc., should not be read as limiting to that function.

[0073] Concerning all methods, the terms “a” and “an” are used to mean “at least one”, “at least a first”, “one or more” or “a plurality” of steps in the recited methods, except where specifically stated.

[0074] It is expressly contemplated that the methods described herein are not limited for the creation of antibodies specific to cancer antigens, but may be used for the creation and selection of target-specific antibodies against any desired antigen, such as without limitation, a viral antigen.

EXAMPLE 1

Detection of Antigens

[0075] For the detection of protein biomarkers various protein assays are available including, for example, antibody-based methods as well as mass spectroscopy and other similar means known in the art. In the case of antibody-based methods, for example, the sample may be contacted with an antibody specific for said antigen under conditions sufficient for an antibody-antigen complex to form, and then detecting said complex. Detection of the presence of the protein antigen may be accomplished in a number of ways, such as by Western blotting (with or without immunoprecipitation), 2-dimensional SDS-PAGE, immunoprecipitation, fluorescence activated cell sorting (FACS), flow cytometry, and ELISA procedures for assaying a wide variety of tissues and samples, including plasma or serum. A wide range of immunoassay techniques using such an assay format are available, see, e.g., U.S. Pat. Nos. 4,016,043, 4,424,279, and 4,018,653. These include both single-site and two-site or “sandwich” assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labeled antibody to a target antigen.

[0076] Sandwich assays are among the most useful and commonly used assays. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabeled antibody is immobilized on a solid substrate, and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labeled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of biomarker.

[0077] Variations on the forward assay include a simultaneous assay, in which both sample and labeled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In a typical forward sandwich assay, a first antibody having specificity for the biomarker is either covalently or passively bound to a solid surface. The solid surface is typically glass or a

polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride, or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g., 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g., from room temperature to 40° C. such as between 25° C. and 32° C. inclusive) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the biomarker. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the molecular marker.

[0078] An alternative method involves immobilizing the target biomarkers in the sample and then exposing the immobilized target to specific antibody which may or may not be labeled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labeling with the antibody. Alternatively, a second labeled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule. By “reporter molecule,” as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e., radioisotopes) and chemiluminescent molecules.

[0079] In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase, and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labeled antibody is added to the first antibody-molecular marker complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of biomarker which was present in the sample. Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity.